REMARKS

Claims 1-6 and 10-19 are pending after entry of the amendments set forth herein. Claims 7-9 were previously canceled without prejudice. Claims 1, 5, 6 and 12-13 are amended to clarify minor informalities; and to rewrite Claims 5 and 6 in independent form. Claims 5 and 6 clarify that the methods of the invention produce both polynucleotides and polypeptides. Claim 1 has been amended to clarify that the exogenous phosphate is an inorganic phosphate, see specification at paragraph 61 for support. No new matter is added and no new issues of patentability are raised.

Claims 5, 6, 12 and 13 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner has noted that Claims 5 and 6 depends from claim 1, which includes three classes of "phosphates": nucleoside monophosphates, exogenous nucleoside triphosphates and exogenous phosphates. Claims 5 and 6 have been amended to clarify that the reference is to exogenous phosphate.

Claims 12-13 were originally filed claims that retained the recitation of "biological macromolecules", which no longer has antecedent basis in Claim 1. The claims have been amended to be consistent with Claim 1, which recites polynucleotides and/or polypeptides.

In view of the amendments and remarks, withdrawal of the rejection is requested.

The methods of the present invention are directed to synthesis of polynucleotides and/or polypeptides in a cell free reaction mix, and particularly to coupled transcription and translations reactions in which both polynucleotides and polypeptides are synthesized. While methods for such synthesis have been described in the art, in a combined transcription and translation reaction the additional requirement for reagents to produce mRNA add to the overall cost of the reaction. With conventional technology, the major reagent costs include the source of chemical energy, enzymes, DNA template, and NTPs. Methods of decreasing these costs while enhancing yield are of great interest.

While the cited art Schulte *et al.* was able to provide for protein synthesis utilizing phosphoenol pyruvate as an energy source (see Table 1), there was a high requirement for this high phosphate energy source, and reasonable levels of synthesis required feeding the reaction with PEP every other hour (see paragraph 0050). Thus, any benefit to the use of nucleoside

monophosphates was negated by the require for a very high input of an expensive high phosphate energy source.

Until the present invention, the field was unable to utilize NMPs as a substrate for mRNA synthesis without incurring high expenses from the cost of the energy source. It was naturally assumed that the contribution of the energy source was in providing high energy phosphate bonds, and thus there was no motivation to look to simple exogenous phosphate as a solution. It is only with the insight of the present inventors that it was determined that, rather than inhibiting by a feedback mechanism, the exogenous phosphate was relieving a shortage and providing for increased yields. For example, one may look to Figure 4 of the present application, which demonstrates that the addition of exogenous phosphate results in a marked increase in yield:

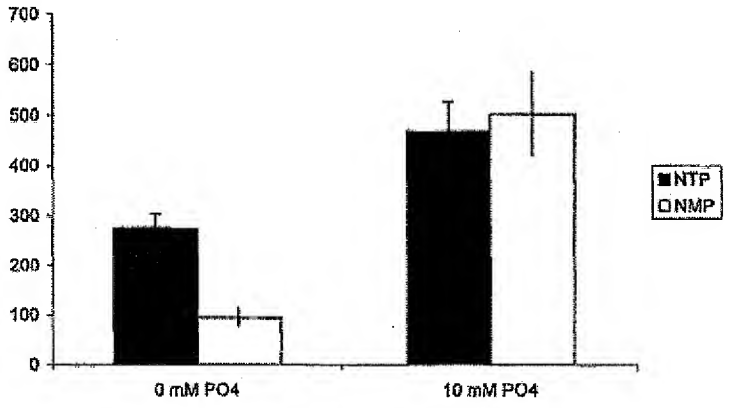


Figure 4

Applicants note, however, that the addition of exogenous phosphate can also inhibit the reaction, and must be provided within an appropriate range. For example, one may look to the evidence provided by Applicants in Figure 2 and Figure 10.

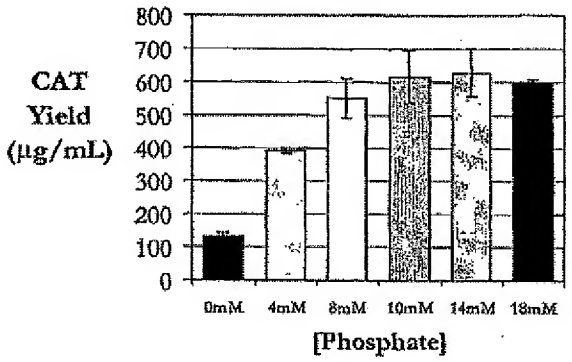
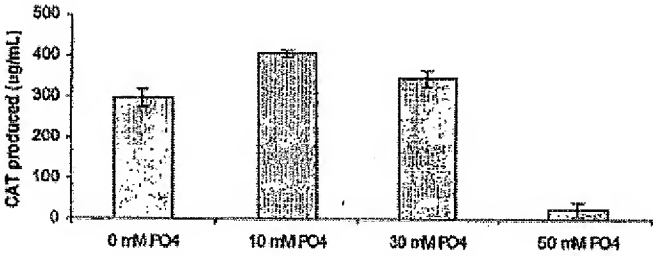


FIGURE 10

Figure 2: Addition of phosphate to cell-free reactions using glucose as energy source



It can be seen from these data that above a certain level the additional phosphate is strongly inhibitory of the reaction.

Through the addition of a suitable concentration of exogenous phosphate, the methods of the present invention allow for production of polynucleotides and polypeptides using a low cost NMP pool and a low cost with the addition of an energy source to supplement synthesis, where the energy source is phosphate free, *e.g.* glucose, pyruvate, glutamate, *etc.* Such energy sources are desirable because they are less expensive relative to sources with phosphate, and particularly sources with high energy phosphate bonds, such as PEP, ATP, glucose-6-phosphate, and the like.

Claims 1, 3-6 and 10-19 have rejected under 35 U.S.C. 103(a) as being unpatentable over Swartz #1 (U.S. Patent # 6168931, issued 2001, in PTO-892, 9/22/10) in view of Schulte et al. (U.S. Patent Application # 2003/0113778, priority date 10/30/01).

These claims are drawn to a method of synthesizing polynucleotides and/or polypeptides in a cell-free reaction mix comprising at least 10 mM of a phosphate free energy source; in the absence of exogenous nucleotide triphosphates (NTPs); and at least 1 mM of exogenous phosphate, which may be provided by potassium phosphate, magnesium phosphate or ammonium phosphate.

Swartz #I teach a method of synthesizing biological macromolecules such as proteins using a reaction mix which comprises nucleoside triphosphates (0.85 mM each of GTP, UTP, CTP, 1.2 mM ATP), and a phosphate containing energy source, phosphoenol pyruvate.

As noted by the Examiner, Swartz #1 clearly teach the use of the exogenous nucleoside triphosphate, ATP as the energy source for translation and transcription, and it is noted by Applicants, the reference utilizes nucleotide triphosphates as a substrate for mRNA synthesis.

The Examiner asserts it would be obvious to one of ordinary skill in the art from the teachings of Swartz #1 that ADP can replace ATP as an alternate energy source, since this ADP/ATP is a cyclic reaction.

Applicants note, first of all, that the present invention is directed to the use of nucleoside monophosphates (for example AMP), not diphosphates (such as ADP), and AMP and ADP are not equivalent molecules. Applicants submit that it is not obvious to substitute AMP for ATP in the absence of a source of high energy phosphate bonds. It is not in any way a simple or equivalent substitution because the bond energy is not equivalent for the phosphates, i.e. the single phosphate linkage to adenosine in AMP is significantly different than the high energy phosphate bonds in ATP.

As is commonly understood in the art, high-energy phosphate refers to the phosphate-phosphate bonds formed when compounds such as adenosine diphosphate and adenosine triphosphate are created. The compounds that contain these bonds include nucleoside diphosphates and nucleoside triphosphates. High-energy phosphate bonds are pyrophosphate bonds, acid anhydride linkages, and the the hydrolysis of these bonds is exergonic under physiological conditions, releasing energy. Applicants note that the energy released by hydrolysis of either ATP to ADP, or ADP to AMP is ΔG [kJ/mol] -36; while the energy released from AMP to A is -12. Clearly there is considerable energy required to create ATP from AMP, and thus the two cannot substitute for each other in reactions due to the difference in energy cost, and the two do not predictably lead to the same results.

As noted by the Examiner, Swartz #1 do not teach the use of the nucleotide triphosphates GTP, UTP, CTP and ATP as substrates to transcribe the DNA template to RNA, and thus does not teach the use of NMPs as substrates.

The Examiner asserts that Schulte *et al.* remedy the deficiencies of the primary reference by teaching a method that converts NMPs to NTPs for *in vitro* synthesis of nucleic acid molecules such as mRNA from a DNA template.

Applicants note the contribution of Schulte *et al.*, but submit that the combination of references does not make obvious the presently claimed invention, which, as explained above, utilizes a phosphate-free energy source and exogenous phosphate. Schulte *et al* do not teach how to usefully synthesize mRNA and proteins from NMPs without repeated feeding of the energy source PEP, which contains high energy phosphate bonds. While Schulte *et al.* aspire to a low cost method, it is not achieved because the high cost of the PEP negates the savings of using NMPs.

Applicants submit that in view of the above amendments and remarks, the presently claimed invention is not made obvious by the cited combination of references. Withdrawal of the rejection is requested.

Claims 1-6 and 10-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Swartz #2 (U.S. Patent # 6337191, issued 2002, cited in PTO-892, 9/222/10) in view of Schulte et al. (U.S. Patent Application # 2003/0113778, priority date 10/30/01).

Applicants submit that the presently claimed invention is not taught or suggested by the cited combination of art. As discussed above, there are energetic considerations that must be taken into account when one attempts to substitute NMPs for NTPs, and one cannot be substituted for the other without providing high energy phosphate bonds by some other means.

Schulte et al. teach the use of NMPs in a synthetic reaction, but do not teach a means of using the NMPs without repeated feeding of PEP, which provides a high energy phosphate bond. And while Swartz #2 teaches methods of polypeptide synthesis using non-phosphate energy sources, such glucose, the method still requires NTPs.

If one of skill in the art were to combine the two references it would not result of the methods of the invention. Rather, it would provide the disappointing results that were obtained when Applicants DID try to combine the two methods, as shown in Figure 10, in the bar with no added exogenous phosphate.

It was only with the inventive input and insight provided by the present application, in which it was realized that an effective concentration of exogenous phosphate was required, that the reaction was able to proceed in a reasonably efficient manner.

Applicants submit that in view of the above amendments and remarks, the presently claimed invention is not made obvious by the cited combination of references. Withdrawal of the rejection is requested.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-337.

Respectfully submitted,
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Date: June 20, 2011

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